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VERNALIZATION

OR

Lyssenko's Method for the Pre-treatment of Seed

BY

R. O. WHYTE, PH.D. and P. S. HUDSON, PH.D.

(Bulletin No. 9 of the Imperial Bureau of Plant Genetics, Herbage Plants and Occasional publication of the Imperial Bureau of Plant Genetics, for plants other than herbage.)

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CONTENTS.

		PAGE.
	Introduction	3
I.	Vernalization of long-day plants	5
	Spring wheat	6
	Technique for vernalizing long-day plants	8
II.	Vernalization of short-day plants	10
	Soya bean and tropical cereals	11
	Technique for vernalizing short-day plants	12
III.	Tuber formation and flowering in potato	14
IV.	Discussion of principles of vernalization	16
V.	Artificial retardation of germination	22
VI.	Biochemical aspects of vernalization	25
VII.	Bibliography	27

INTRODUCTION.

The new process* elaborated by the workers at the Odessa Plant Breeding Institute has been defined as " early sowing which is carried out in the granary." Thus all the advantages of early sowing in spring crops are attained under conditions where actual sowing in the field would be impossible owing to the snow cover, low temperature and the like. The Soviet workers have produced a series of publications describing the experiments on which the method is founded ; the object of the present bulletin is to give a brief outline of the general conception and methods so that the reader may form his own opinion as to the merits of the process and its possible application in general agricultural practice. The material has been considerably abbreviated, but a list of the articles on which it is based is included in the bibliography and English translations of any of these are available on application to the Bureaux.** The work is at present only in a preliminary stage and extensive investigations are in progress. It is hoped to publish further communications on the subject as details are received. The authors wish to express their appreciation of the valuable assistance given by correspondents of the Aberystwyth Bureau at Odessa and Lugowaya.

(*) The Russian word "Jarovizatzia," referred to in German publications as "Jarowisation", has here been translated by the latinized equivalent " vernalization " in consultation with the School of Slavonic Studies, London, and Miss M. V. Cytovich, of the Imperial Bureau of Animal Genetics.

(**) See also special article by A. Favorov in *Herbage Reviews*. Vol. 1. No. 1. 1933.

I.—VERNALIZATION OF LONG-DAY PLANTS*

Vernalization has in view the shortening of the period of time necessary for a plant sown under ordinary field conditions to reach the fruiting stage. The length of the vegetative period of the plant, which is frequently of great importance when this is sown in different regions, is also shortened by this process.

As is well known, plants may be sub-divided into three types, long-day plants, short-day plants and plants which do not react to differences in length of day. One of the points emphasized by the Odessa workers is that the vegetative period is not a constant characteristic of a plant but depends on the influence of the external conditions of the region in which it is grown. Certain combinations of these conditions tend to shorten the vegetative period. Those external factors which produce the shortest vegetative period vary for different plants and even for different varieties of the same plant, e.g. in wheat, winter and spring varieties shew marked differences in their requirements as regards climatic factors. Moreover the reaction of the plant is determined not by individual factors, but by a set of factors acting together ; any one of these individual factors may, nevertheless, succeed in hindering development.

Under artificial conditions in a greenhouse it is possible arbitrarily to accelerate or retard reproduction by varying the conditions of light, darkness, temperature, humidity, etc., according to the special requirements of each plant. Under ordinary field conditions this is not the case, and the vegetative period of a plant depends mainly upon the natural conditions of the regions in question. Hence in any particular area only those plants whose vegetative period is such that seed formation falls within the limits of the external factors characteristic for the region are suitable for cultivation.

The essence of the method of vernalization is the elimination of the effect of natural geographical and climatic factors limiting the introduction into field cultivation of any desired plant. Before discussing its practical application or prospects of its utilization, it is necessary to discuss the fundamental principles underlying the hypothesis on the basis of which the method of vernalization has been worked out for various plants.

The Odessa workers make a clear distinction between the phenomenon they refer to as *growth*, namely increase in weight and size of the plant, and the phenomenon they refer to as *development*, whereby they understand the transition of the plant to successive phases, in other words, a qualitative change in the nature of the plant. In the present work only that change which concerns the transition to the reproductive stage is involved. The complex of factors conditioning the reproduction of the plant is not, it is maintained, always identical with those conditioning the growth processes.

"It has been experimentally proved that the time necessary for the completion of the processes conditioning reproduction of cereals does not depend upon the size and age of a plant, but only upon genotypical peculiarities of the plant organism and its environment. Thus winter wheat, whether at the stage in which the embryo has just started growth but has not broken the seed coat, or whether it is a well-developed plant, requires for transition to the reproductive stage the influence of the same factors acting during the same length of time. The length of time may vary according to changes in the external medium, but in this case it will vary in a similar manner for the embryo and for the adult plant. Thus the central statement of the hypothesis is as follows :—*the processes conditioning the sexual reproduction of cereals may occur not only in green plants but also in a seed with an embryo which has just commenced development but has not broken the seed coat, i.e. the material to be sown.*"

(*) Lyssenko (13 and 14).

They emphasize the fact " that the transition to the reproductive stage is not only independent of the size and age of the plant, but is not correlated in time with growth. The plant may change qualitatively in a required direction without continuing to grow and vice versa, the plant may grow and yet not effect the required qualitative change. The processes of vernalization are independent of growth but connected with the environment; if the external condition be favourable both for growth and qualitative change in the plant, the plant will grow and pass through the stages preparatory to reproduction. *The fact that the process of preparation of a plant towards reproduction may occur in the embryo and may be separated in time from the growth of the plant makes possible the practical application of the method of vernalization.*"

In order to carry out the process of vernalization the seed material must be brought into this necessary condition. This is effected by forcing the seed to grow and then artificially stopping the growth, after which the seed is subjected to the influence of the appropriate external factors before sowing. Thus the sowing material, represented by this pre-treated seed, may have the appearance agronomically of seed, but from this new point of view it should be regarded as seedlings.

" Vernalization is the influence of the complex of factors corresponding to the plant in question which make possible the occurrence in the seed material of those processes which determine the transition of the plant to the reproductive stage. The work with cereals is only a special case of the general problem of artificial acceleration of reproduction in agricultural plants sown under ordinary conditions. Hence vernalization of different plants differs only in the complex of factors under the influence of which the plants are placed."

In order to explain accurately the way in which vernalization of cereals may be adopted in agricultural practice, the Odessa workers emphasize the fact that there is no distinct limit clearly dividing winter and spring plants one from the other. All varieties differ from one another merely in the degree of " winterness." Usually by winter varieties one understands those varieties which when sown in the spring do not come into ear the same summer. This distinction however is, in the opinion of the Odessa workers, unfounded. This was illustrated by an experiment in which different varieties were subjected to different dates of sowing. Certain varieties did not ear even when sown early, others failed to do so only when sown late. Almost all spring varieties failed to ear when sown at the end of May and thus, as pointed out by Lyssenko, in these circumstances behaved as winter varieties. In similar trials with barley, sowings of the same variety made every other day up to the 12th March all produced ears. On this basis the variety would be regarded as a spring type. All sowings after March 12th, however, failed to produce ears. Similar behaviour can be observed in any variety, but the particular dates will differ for varying varieties.

SPRING WHEAT.

The main difficulty in cultivating spring wheats in the semi-arid zones of the Ukrainian Steppes is high summer temperature which damages the wheats just at the time they are coming into ear. Thus for these conditions the chief requirement in a spring wheat is early ripening; a variety may be superior in all other respects and yet may suffer damage owing to its lateness. By overcoming the effect of this external climatic obstacle by the use of vernalization it is possible to produce a large group of varieties considerably earlier than the local early wheats and from which it will be possible to find an early variety suitable for any given region.

Some account may now be given of trials carried out in the Ukrainian Institute of Plant Breeding at Odessa in 1931; 1,260 pure lines, mostly *durum* wheats, were collected from various regions of Soviet Azerbaijan. These wheats when sown in the Steppes of the Ukraine are exceptionally late and fail to give a crop owing to heat damage before ripening. The wheats were sown on the 21st April, both in a vernalized and untreated state, certain *vulgare* and *durum* spring wheats collected from 31 regions of the Ukraine being sown at the same time. The Ukrainian group first began to

ear on the 15th June (*lutescens* 062 of Saratov Station) and finished on the 21st June (Kochin's Arnautka). When the latest of the Ukrainian varieties had completed earing, 4.2 per cent. of the Azerbaijan wheats were in ear. By the 1st July, i.e., 10 days after the last Ukrainian wheats came into ear, only 11.1 per cent. of the Azerbaijan wheats had eared. Hence it is obvious that if spring wheats, more especially the *durum* varieties, are very frequently damaged by heat, there is no point in attempting to replace them by still later varieties from Azerbaijan.

After the application of vernalization an entirely different result was obtained. The Azerbaijan wheats when sown after treatment proved to be earlier than the earliest Ukrainian variety. By June 15th, i.e. at the flowering date of the early Saratov 062, 66 per cent. (830 varieties) of the vernalized Arzebaijan varieties had eared. Of these vernalized wheat varieties, 333 eared 5 days earlier than Saratov 062, some of them even 8—10 days earlier. The untreated Azerbaijan wheats gave no seed or only very weak specimens, while vernalized wheat produced well-developed grain considerably superior to the grain of Ukrainian wheats of the same sowing.

A variety test was set up with two varieties of Azerbaijan wheat with 8 replications in order to make an accurate comparison of the yields. Both these varieties were sown in the vernalized state in 1930 in a small collective nursery. The results are shown in the following table.

TABLE I.

Name of Variety	Sowing	Ear formation.	Acceleration (—) or retardation (+) in earing in days as compared with Girka 0274	Yield in centners* per ha= 2.5 acres.	Yield as percentage of Girka 0274
Azerbaijan. *erythrospermum* 534/1 vernalized ..	11th April	5th June	—9	7.4	111.7
do. untreated ..	,,	1st July	+17	0.3	4.7
Azerbaijan. *ferrugineum* 1316/8 vernalized. ..	,,	12th June	—2	8.9	141.1
do. untreated. ..	,,	1st July	+17	0.5	7.9
Odessa Girka 0274 (*milturum*)	,,	14th July	0	6.3	100

*Centner in Germany and Sweden = 100lb., but according to official Soviet data (subject to confirmation) the Centner in the U.S.S.R, = 200lb.

As may be seen, even these two varieties selected from a small collection which would have been unsuitable for Odessa gave after vernalization an increase of yield over the recently selected variety Girka 0274 (*milturum*). Taking Girka as 100, *erythrospermum* 534/1 gave 111.7 per cent. and *ferrugineum* 1316/8 gave 141.2 per cent. when vernalized, whereas when untreated they gave no yield, earing too late and suffering from scorching. It should be observed, however, that from May 1st to June 30th only 19.2 mm of rainfall were recorded at the Institute, while on June 30th there was a heavy fall of rain (46.8 mm.). This rain could be used only by those varieties which ripened in mid-July, with the exception of *erythrospermum* which on July 2nd-4th was already in the wax-ripe stage. In spite of the fact that this variety grew almost without rainfall, its yield was superior to that of Girka, which had ripened normally 15 days after the rain.

These results justify the assumption that it is possible by the method of vernalization to select still earlier varieties of both *durum* and *vulgare* wheats which would be more suitable for semi-arid regions. Even wider prospects may be envisaged by sowing the world collection of wheat in the vernalized state. The U.S.S.R. National Commissariat of Agriculture decreed that vernalized samples of these wheat varieties should be tested in 1932.*

The above statement shows that the vernalization of cereals aims not, as many think, at the substitution of autumn sowings of winter varieties by spring sowings of the same varieties, but at the replacement of the local low-yielding late spring wheats. This replacement should be effected by the use of winter and semi-winter varieties as well as of late spring varieties, which three classes include the majority of wheats, and from which it will be possible to select the variety suitable for a given region.

In discussing the relation between vernalization and breeding, Lyssenko stresses the fact that vernalization is not in any way antagonistic to breeding but is actually complementary. Vernalization without plant breeding and genetics is quite impossible. All the Odessa results are based primarily upon the varying reaction of varieties to external factors. " Inasmuch as the workers with vernalization utilize genotypical differences in varieties," says Lyssenko, " plant breeders should employ vernalization."

In Lyssenko's opinion, plant breeders when using the method of vernalization will be able rapidly to select the required variety. It is maintained that as a result of breeding work vernalization will become superfluous. During the first 5—10 years, however, until new varieties are introduced and multiplied, the new method must play its role in the increase of agricultural yields, more especially of *durum* wheat. At the present time breeding methods are being re-modelled with a view to their acceleration and vernalization offers a valuable means of obtaining several generations of winter wheat in the greenhouse within the same year.

All the facts relating to wheat are true also for other crops, but the experimental data so far collected are not extensive. Lyssenko is confident, however, that the same phenomenon will be observed and that late varieties which at present do not yield seed, will be duly transformed into early varieties when treated by the methods of vernalization.

TECHNIQUE FOR VERNALIZING LONG-DAY PLANTS.**

The report refers to *Triticum durum.*

The following rules must be borne in mind :

1. Vernalization can be effected only in those seeds in which the embryo has at least begun to develop. Odd seeds should have already sprouted.

2. The best temperature is between 1° and 5° C. in the heap of grains. Vernalization cannot be effected at 0° or below.

3. The grains should be moist, a moisture content of 45-50 per cent. calculated on the basis of absolutely dry material. With a lower moisture content the vernalization is extremely slow and with higher the seed begins to germinate and this may cause difficulty in subsequent sowing.

4. Vernalization must be begun not less than 10-15 days before sowing. A thermometer must be placed in the centre of the pile and according to its readings the temperature must be regulated.

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*Accordingly on 16/4/32, some 6,300 samples of wheat were sown in the vernalized and untreated states in Odessa. It was noted that the various ecological types showed different requirements as regards the complex of factors necessary for accelerated vegetation and straw formation.

** Bibliography (1).

Preparation for Vernalization.

1. *Choice of Place.*
Indications are given of the type of building suitable. Ventilation is essential to prevent dampness and the consequent danger of moulds. For every square metre of floor 75 lb of grain can be treated, the grain being put at a thickness of 15-18 cm.

2. *Apparatus necessary.*
This is enumerated in the original articles.

3. *Determination of the amount of water necessary.*
Grain ordinarily stored in a dry granary contains 13 per cent. moisture, hence to bring this up to 50 per cent. it is necessary to add 33 litres of water to each 100 kg. of grain, in other words water at the rate of one-third the weight of grain taken. For each per cent. moisture in the grain 1 kg. less water is needed.

The water is applied to the pile of grain in 3 successive applications. The room should be at 10-12° if possible, to secure a more rapid absorption.

4. *Moistening the grain.*
Detailed instructions are given for carrying out this.

5. *Germination of the grain.*
Four hours after the completion of the final moistening the swelling grain is spread out in layers of 20-25 cm. on the floor, at a temperature not higher than 15° C ; if lower it will have to be in thicker layers and covered. It must be well stirred after 8-10 hours. After 24 hours all the embryos have begun to grow. As soon as any are observed to have pierced the grains vernalization should be begun.

Vernalization.

The first essential is to reduce the temperature to 3° C ; below this vernalization is negligible (at 0° or below no vernalization occurs) and above 5° the danger of mould and germination is too great.

The temperature reduction can be attained at night by opening the doors and windows, which are tightly closed again during the day. The temperature must never be allowed to exceed 5°. Spring wheat is vernalized for 12-15 days from the reduction in temperature. A specimen table for keeping temperature results is given.

The grain must be stirred every day. After 10-15 days the grain is vernalized and is ready for sowing. It is weighed, so as to calculate the amount to sow per hectare to give the same number of grains as the control sown with untreated seed.

If it cannot be sown at once it must be dried.

Calculation of the hours of labour required to vernalize one ton of grain.

Total working hours calculated as 356.

II.—VERNALIZATION OF SHORT-DAY PLANTS.

Having considered the phenomenon of vernalization as it affects long-day plants such as wheat and crops of the temperate zones in general, it may be of interest to study the behaviour of a completely different group of plants, namely those associated with tropical and sub-tropical regions, where conditions of light, temperature, etc. are entirely different. As types of such plants Lyssenko and his co-workers (2, 3, 12) have chosen maize, millet, sudan grass, sorghum and soya bean. All these plants share the common characteristic of requiring a combination of high temperature and short daily period of light for reproduction. Whereas under continuous illumination wheat and plants of similar latitude accelerate their reproduction, the tropical group under such conditions may fail to enter the reproductive stage at all. This more than any other single factor is an obstacle to the extension of their cultivation into more northern latitudes, in the case of some plants even into North Ukraine. On the other hand, when this obstacle is removed by artificially curtailing the period of illumination, many varieties of such a southern plant as soya bean can be made to ripen even as far north as Leningrad.

This was the basis for a series of further experiments carried out at Odessa in which the principle of vernalization was applied to these short-day plants*

To determine the requisite factors for the vernalization of seed of short-day plants it was necessary to determine (1) what external conditions are required by a plant for its rapid transition to the reproductive stage, (2) what individual factors must be included in this set of external conditions and (3) which climatic factors favour, and which retard reproduction in short day plants.

When these questions have been ascertained and the optimum dose of each factor in relation to the other factors operative has been determined, it will then be possible to subject the seed to the necessary treatment.

As in the case of wheat a clear distinction has been made between growth processes and reproductive processes. Short-day plants require light for the processes of growth and darkness for reproduction. Emphasis has been laid on the fact that these influences are exerted by light and darkness themselves, and not necessarily by an alternation of the two.

It has been claimed that "by applying the influence of the appropriate complex of external conditions on the sowing material of these plants it is possible to force them to set seed under field conditions not only under long day, but even under continuous day, i.e. in the entire absence of night. Seeds of millet which had been treated for five days by the appropriate factors (humidity, temperature, darkness and aeration) were then sown, some under ordinary field conditions and some under continuous illumination (electric lamps at night). Controls of untreated seed of the same varieties were also sown under both these conditions. The treated plants under ordinary field conditions flowered sooner than the control under the same conditions. Treated plants under continuous light reached maturity, while the control under the same conditions did not come into ear. These experiments shewed that a short-day plant like millet may on vernalization accelerate its reproductive phases both under conditions of long day and of continuous day."

It is important therefore to note that after the application of all the requisite darkness in one dose to the seed, *the resulting plants require no further period of darkness to enable them to pass through the reproductive phases.*

(*) By analogy the same word vernalization is applied to this phenomenon of accelerated reproduction as observed also in short-day plants.

SORGHUM.

Sown 10th September. Photographed 29th December.
Left=Control. Right=Plant which received 10 days pre-treatment.

The processes for which darkness is necessary have already taken place at the time of vernalization and therefore the growth of the resulting plants is independent of the darkness factor and the plants can be grown under any type of illumination. Moreover, it is claimed that there is no antagonism between vegetative growth and reproduction and that by the application of vernalization both accelerated reproduction and increased production of dry matter can be effected side by side. In this respect Lyssenko's principle is clearly distinguished from photoperiodism in the usual sense. The greater accumulation of dry matter in the vernalized plants is due to the increased photosynthesis resulting from their exposure to a period of illumination considerably longer than that possible under ordinary methods of photoperiodism.

SOYA BEAN AND TROPICAL CEREALS.

Results similar to the above have been obtained in preliminary experiments with soya bean, Sudan grass, sorghum and maize. In every case it was possible to produce the desired results only when the degree of darkness was applied in conjunction with suitable degrees of the other three factors temperature, humidity and aeration. It was found that darkness alone, when these other factors were not suitably adjusted, was not capable of producing the required effects.

In order to demonstrate this point, Dolgushin* laid out tests with 16 pots of millet seedlings. These were sown on the 16th July and divided into the following four groups :—

A.—daily darkness from 6 p.m. to 7 a.m., with the temperature varying between 10 and 12°C. ;
B.—illuminated in the open by electric light at night ;
C.—darkness in the open with the natural alternation of day and night ;
D.—darkness at the same rate as group A, but with a temperature of 22°C.

After August 4th certain pots of each group were transferred to (a) continuous illumination, and (b) normal growth conditions. Those treated by darkness throughout the trials with a temperature of 10—12°C., together with the plants of group A (No. 856) which had been exposed to continuous illumination from August 4th onwards, and also the plants of group B (No. 843) which had received continuous illumination throughout the trial, remained throughout in a vegetative state of development. That is to say, darkness with a temperature of 10—12°C., like constant illumination, is a condition under which the reproductive development of millet is impossible.

Plant No. 854 of group A, which had been growing since August 4th under normal conditions, flowered at the same time as plant 848 which had received constant illumination up to August 4th, and had then been exposed to normal day conditions. This result confirmed the statement made concerning 856, i.e., that darkness accompanied with a temperature of 10—12°C. has no effect whatsoever. Plant No. 858 of group C, which received the normal length of day throughout the trial as well as plant 857 of the same group under normal day for the first 20 days up to the 4th August and then by continuous illumination, flowered a few days before plant No. 854 of group A which had been treated with 20 days of darkness at a temperature of 10—12°C. and then exposed to normal daylight with a somewhat higher temperature. Plants 845 and 855 of group D, which were grown in darkness at a temperature of 22°C. in the greenhouse for the first 20 days and then exposed to continuous day and normal day respectively, flowered about 12 days earlier than plant 854.

These results amply illustrate the dependence of the process on a combination of several factors and not one factor acting alone. For example, darkness at a temperature of 10—12°C. was without effect whereas when combined with a temperature of 22°C. it produced the maximum acceleration of reproduction.

Experience has shewn that both in the northern cereals and in the tropical group the conditions necessary for the vernalization of different species and even different varieties may differ considerably.

The experiments on short-day plants are to be regarded as preliminary in nature and the precise duration of the treatment of the various species and varieties has not yet been determined so accurately as in the case of wheat.

*Bibliography (3 and 8).

TECHNIQUE FOR VERNALIZING SHORT-DAY PLANTS*

General Part.

1. Seeds must necessarily be made to germinate, at least until cracks appear on their coats.

2. Seeds so treated must be kept in darkness as under :—

millet	for 5	days	at a	temperature	of	25-30°C.
sudan grass	„ 8-10	„	„	„	„	„	25-30°C.
sorghum	„ 8-10	„	„	„	„	„	25-30°C.
maize	„ 10-15	„	„	„	„	„	20-30°C.
soya bean	„ 10-15	„	„	„	„	„	20-25°C.

3. In the course of vernalization, it is necessary to watch carefully both the temperature of seed under vernalization and the rate of seed germination, as overgrown seeds cannot be drilled.

4. Vernalization should be begun as many days before sowing as are required to complete vernalization of the crop in question, e.g.

for millet 5 days before sowing.
„ sudan grass
„ sorghum } 8-10 days before sowing.
„ maize
„ soya bean } 10-15 „ „ „

5. Seeds to be vernalized should be thoroughly cleaned from any impurity, broken seeds, etc., and their germinating capacity ascertained.

Soaking Seeds.

1. In order to cause the embryo to start growth and yet to prevent it from overgrowing, it is necessary to treat seed with the following quantities of pure water per 100 parts by weight of seed.

100 parts of	soya bean	seed	(by weight)	to	75	parts	of	water.
„ „ „	maize	„	„	„	30	„	„	„
„ „ „	millet	„	„	„	26	„	„	„
„ „ „	sudan grass	„	„	„	26	„	„	„
„ „ „	sorghum	„	„	„	26	„	„	„

These proportions should be carefully verified by weighing. The procedure to be adopted in soaking is as follows : the seed is placed in an appropriate vessel into which the required quantity of water is poured ; the seed should be stirred at frequent intervals, thus allowing it to absorb water uniformly ; in approximately 24 hours all the applied water will be entirely absorbed. The temperature of the room where soaking is done should be about 10-15°C.

Vernalization.

As soon as the seeds to be vernalized have absorbed all the water, they should be either strewn on the floor or put in sacks The thickness of seed layers on the floor should be controlled in harmony with the temperature inside the building. The temperature in the seed layers should be as under :

for	maize	20-30°C.	during	10-15 days.
„	millet	25-30°C.	„	5 „
„	sudan grass	25-30°C.	„	8-10 „
„	sorghum	25-30°C.	„	8-10 „
„	soya bean	20-25°C.	„	10-15 „

Temperature should be controlled and recorded once or twice daily throughout seed vernalization. If the temperature be lower than required, the layers of seed should be made thicker, while if the temperature be higher than required, seed layers should be made thinner and aired at night. It must be borne in mind that during vernalization, seeds should be carefully covered so as to keep out any light.

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* Bibliography (1 and 2).

SUDAN GRASS.

Sown 10th September. Photographed 29th December.

Left = Control. Right = Plant which received 10 days pre-treatment.

The control of seed germination is also essential. If seeds grow too rapidly their layers should be made thinner and dried somewhat and vice versa. The degree of moisture should be such that seed germinates during the required period of vernalization and yet the resulting seedlings are not longer than 1-2 mm. for sorghum, sudan grass, millet and soya bean, and less than 2-3 mm. for maize.

To prevent the appearance of mould on seed under vernalization, seeds should be stirred as often as possible, at least once daily.

The conditions required for vernalization of the crops in question may be summarized in the following table :—

Crops.	Quantity of water per 100 parts of seed (by weight).	Number of days (24h.) required for complete vernalization.	Temperature inside seed layers or in sacks during vernalization.
Maize	30	10—15	20—30 °C.
Millet	26	5	25—30 °C.
Sudan Grass	26	8—10	25—30 °C.
Sorghum	26	8—10	25—30 °C.
Soya bean	75	10—15	20—25 °C.

Notes on Sowing.

In order to confirm the results achieved by vernalization both with regard to the time of maturity and yield (whether for seed or hay), it is necessary to sow control (untreated) seeds at the same time, and in plots as far as possible identical. It is necessary to calculate the amount to be sown per hectare to give the same number of grains as the control sown with untreated seed.

III.—TUBER FORMATION AND FLOWERING IN THE POTATO.

The distinction between the processes of growth and reproduction has already been stressed in connection with the acceleration of reproduction in cereals and other plants. The potato on the other hand offers an example of a plant in which the main agronomic interest is centred in the vegetative activities. In this crop any improvement will lie in the direction of an acceleration of tuber production coincident with an increase in tuber yield. To achieve this however it is necessary to study the relationship of tuber formation to the general progress of development and growth of the potato as a seed plant.

Trials were commenced by Lyssenko and Dolgushin (16) at Odessa on February 23rd, 1932, by growing four varieties of potatoes in pots in the greenhouse under (a) continuous day (electric illumination by night), (b) 12-hour day, and (c) 9-hour day. The actual results obtained in these trials correspond entirely to those reported by Rasumov (18) in his work with wild potatoes. Plants which were grown under continuous day were early in commencing growth and flowered at a fairly rapid rate. Plants grown under a shortened day of 9 or 12 hours had a stocky aspect with shortened stem internodes and did not flower. On the 49th day plants were taken from the pots. Tubers were nearly absent on plants grown under continuous light, whereas they were formed in very large numbers on the other two types. To explain his analogous results, Rasumov states that " all plants in the trial without exception shew most vigorous vegetative development and generally flower most abundantly under a long day. A short day, although weakening the vegetative development of the plant, gives at the same time the optimum conditions for tuber formation."

In certain groups of potatoes used by Rasumov, it was observed that abundant tubers were formed when grown under shortened days, but as soon as these plants were transferred to long days there was not only cessation of growth, but even disappearance of the tubers. On page 21 of Rasumov's paper it is stated that " the influence of the long day on *Solanum demissum* is so strong that it excludes any possibility of after-effect of the short day, as may be seen from the following : plants while under short day acquire peculiar short-day habits such as rosette formation and the absence of flowering. As soon as these plants are transferred to long day, vigorous growth begins and plants with well-developed stems and abundant flowers are produced," and later on the same page, " if even in short-day conditions before transference to the long day some tubers were formed, they do not develop and may be partly converted into stolons."

Therefore, the after-effect of short-day on tuber formation which Rasumov postulated is very weak or altogether absent. The error in suggesting the existence of an after-effect of short day on potatoes is, in Lyssenko's opinion, due to the fact that " Rasumov and many other research workers consider tuber formation to be a function of short-day conditions."

In analysing the results obtained both by Rasumov and himself, Lyssenko reaches somewhat different conclusions. In his view the short day has no direct relation to tuber formation in spite of the fact that under such conditions the tubers of four varieties in the trials were formed much earlier than those of the same varieties grown under continuous light. Whereas Rasumov bases the interpretation of his results on the statement that the same plant may be influenced both by a long and short day and later by the after-effect of both long and short day, in Lyssenko's opinion it is possible to find an after-effect in the sense of accelerated growth only in the case of the influence on long-day plants of long day conditions and short-day plants of short day conditions ; an after-effect may be found only when the mutual relationships between the development of a plant and the external media are such that the plant organism undergoes a qualitative change.

Lyssenko and his co-workers again attach great importance to the distinction between growth and reproductive development as exemplified respectively by flowering and tuber formation in the potato. Flowering in the potato is prevented entirely by short-day conditions and the potato is thus to be regarded, from the ordinary viewpoint, as a long-day plant.

" The initial starting point in the individual development of a seed plant is the seed, and the final state is also the seed. Between these two points lie a large number of different stages of development." By the action of the appropriate external conditions plant development may be arrested

at any stage, but this retardation of development does not mean the retardation of plant photosynthesis or increase of the plant mass. Since the potato plants grown under short-day conditions do not progress towards reproduction, it may be said that the short-day is the " brake " in the development in the sense of the progress towards reproduction. The short-day, however, combined with the complex of other factors used, such as temperature and humidity, does not act as a brake to the photosynthesis of the plant. Directly analogous with the behaviour of the potato plant under short-day conditions is the behaviour of crops of spring or vernalized winter wheat under the same conditions. It is known that wheat grown under short day does not ear as its development is hindered by external factors, whereas photosynthesis may progress under short-day conditions, other conditions being favourable. The products of assimilation accumulated by plants cannot be used for development, that is the passage to the final stage (seed formation) ; therefore they are spent in the case of wheat for increased leaf formation, whereas in potato they are partly or entirely used for stolon and tuber formation. If the experimental plants of wheat and potato be transferred from short to long day, then wheat ceases to form leaves and produces straw, i.e., it continues its development, whilst the potato ceases to form tubers and proceeds towards flowering. If conditions be favourable for accelerated development, then the products of photosynthesis accumulated by the plant at that time can be entirely used up while the deposits in the tubers can also be drawn upon. This is how Lyssenko explains the case of reduction in size of tubers formed under short-day conditions when removed to long days. Both in Rasumov's and Lyssenko's trials the short days hindered plant development, but did not stop photosynthesis, and the products of this process were used for tuber formation. In these cases, therefore, although tuber formation might be regarded as a result of the direct influence of short day, it was actually due, according to Lyssenko, to the retardation or possibly to the absence of plant development.

Practical experience of potato cultivation in northern latitudes has shewn that long days favour rather than hinder tuber formation as compared with southern short days. Lyssenko's explanation of this is that the potato as a long-day plant develops quicker under a long day and still better under continuous light. If this development proceeds rapidly under the stimulation of a long day, then in most cases all the products of photosynthesis are used exclusively for plant development and not for tuber formation. Plants when grown under uninterrupted light mature very rapidly and do not form tubers, as all the products of photosynthesis are used for development of stems and flowers. This has apparently occurred in potato cultivation in northern latitudes. Immediately after the potato seedlings appear, they develop rapidly through the preparatory stages and proceed to flower and to form fruit if the flowers are not lost. When the cycle of development is completed by fruit formation, the nature of the potato plant is such that it does not perish, but continues to assimilate and to use the products of assimilation entirely for tuber formation. In this way Lyssenko explains tuber formation in northern latitudes. The hypothesis is confirmed by trials at Odessa, where potatoes cultivated under constant illumination formed flowers and then commenced abundant tuber formation. Under this constant illumination, tuber formation began earlier than in the plants of the same variety grown under normal conditions, while in addition the yield of tubers by weight per plant grown under constant light was much higher. The yield per plant was :—constant illumination= 374 gr., normal day=260 gr., and 9-hour day=250 gr. Therefore, in order to accelerate tuber formation of a potato under field conditions, neither preparatory development of the plant nor flowering must be hindered.

TECHNIQUE FOR VERNALIZING POTATOES.

The essence of vernalization of potatoes is the application of continuous illumination for a few weeks before planting in the field. The method recommended by Lyssenko and Dolgushin is as follows :—

Potato tubers are threaded on wire or string, 2.5 kg. of large tubers being placed over a length of one metre, and hung vertically in a greenhouse 20-30 days before planting into the field. All the eyes of each tuber should be uniformly illuminated by sunlight during the day and strong electric light by night. Under such treatment the buds grow rapidly, but the young growths are kept short because of the light and the dryness of the air, and there is no fear of breaking them during removal to the field. These bundles may be hung in any room where there is light and a temperature of 15-20°C.

IV.—DISCUSSION OF THE PRINCIPLES OF VERNALIZATION.

The phenomena of photoperiodism in general have received considerable attention from plant physiologists, and the after-effect observed by S. A. Eghis working on soya beans near Leningrad was one of the first results which seemed to give promise of the practical application of photoperiodism. The phenomenon as interpreted by Eghis is that if, for example, short-day plants are grown first under several long days and are then exposed to the influence of short days, the development of their reproductive organs is retarded because of the accumulation, while under long-day conditions, of certain factors inhibiting this progress. By exposing the plants to the influence of the short day, such factors are eliminated and the plants thus receive a stimulus towards reproductive development. If a certain number of short days be applied, followed by exposure to long days, the plants receive the stimulus towards reproductive development from the start and consequently reproduction is possible and may even be accelerated.

The interpretation of these phenomena put forward by Maximov, Rasumov and others is that the acceleration or retardation in the formation of reproductive organs bears a certain relation to the number of long and short days which the plant receives ; from his studies on the effect of long and short days on the date of flowering of short-day plants, Rasumov concludes that short days have a greater after-effect on these plants than long days. According to the number of short days received early in development, reproduction was proportionately accelerated when the plants were later grown under normal long-day conditions.

The application of the principle of the after-effect in the ordinary sense is limited in practice by one important consideration. Lyssenko states that he himself has never observed either in practice or in the literature, cases in which material previously treated by growing in an artificially regulated day has been sown to any extent. He explains the failure to apply the after-effect of photoperiodism to short-day plants by the fact that the phenomenon is easily detectable by physiologists only in those plants which in practice are usually sown direct into the field and not transplanted. Plants which are grown by transplantation usually shew a very small after-effect, since the seed material sown in normal conditions at the end of the winter and beginning of spring grows under the influence of the natural short day.

Moreover, certain investigators who have studied the phenomenon of the after-effect, have, on the basis of their observations, come to the conclusion that the after-effect does not occur in all plants which react to photoperiodism. For instance, Rasumov has observed that the majority of tuber plants in his experiment (including certain varieties of potato) which react very markedly to photoperiodism are almost devoid of and shew hardly any expression of the after-effect.

Dolgushin (8) carried out two trials on similar lines, the first on the after-effect of short day, the second of long day, but again came to somewhat different conclusions. In the first trial, 24 pots of millet (short-day plants) were divided into two series. The first series was exposed to normal day conditions of alternating day and night, whereas the second series was placed under continuous day, electric light being supplied during the dark hours. On every third day two pots, one from each series, were exchanged. Thus at the end of the trial two series were obtained : the plants of the one series had received 3, 6, 9 . . . . . up to 30 normal days respectively, and had then been under constant illumination, whereas the plants of the other series were exposed to the same quota of continuous day, and were then grown under normal conditions. Two control pots in each series were grown under unchanged conditions throughout the trial. The results of the first series of experiments are tabulated in Table 2 :

TABLE 2.

Number of normal days.	0	3	6	9	12	15	18	21	24	27	30	Normal days only.
Date of exsertion of panicles	–	–	–	–	22/8	21/8	18/8	20/8	23/8	22/8	22/8	20/8
Number of days from germination to exsertion of panicles.					24	23	20	22	25	24	24	22

It may be seen that the plants which received nine normal days or less remained in a state of vegetative development throughout the trial. Plants which received 12 or more normal days flowered almost simultaneously, no matter how many days had passed since they were removed from normal to continuous illumination. " The fact that millet plants which received nine normal days or less did not flower until the end of the trial indicates that millet for its transition to the stage of reproduction requires a certain quota of darkness contained in a certain number of 24-hour days (in this case 12). Any increase in this quota of darkness does not accelerate the transition of plants into the reproductive stage. If the millet plants do not receive a sufficient quota of darkness (in this case nine or less normal days) they will remain indefinitely at the vegetative stage when transferred to conditions of continuous illumination. By supplying to these plants the amount of darkness they lack, we may thus force them immediately to commence the development of reproductive organs. It might be assumed that darkness is one of the factors whose presence is required for the transition of short-day plants towards the reproductive stage."

It is still possible in Dolgushin's opinion to speak of the after-effect of photoperiodism, though not in the sense of removing by shortened day whatever " brakes " may be supposed to have arisen in the plant itself or of supplying a stimulus given by the short day towards the reproductive stage, " but in the sense of the presence or absence of certain external factors in whose presence the processes conditioning reproduction may occur."

The figures of the second series of experiments in the first trials are presented in Table 3 :

TABLE 3

No. of continuous days.	0	3	6	9	12	15	18	21	24	27	30	Continuous day only.
Date of exsertion of panicles	20/8	24/8	26/8	28/8	30/8	2/9	5/9	7/9	9/9	10/9	13/9	—
Delay in exsertion (in days) ..	–	4	6	8	10	12	15	18	20	21	24	—
Number of days from germination to exsertion. ..	22	26	28	30	32	35	38	40	42	43	46	—
Number of normal days passed before exsertion	22	23	22	21	20	20	20	19	18	16	16	—

Analysing the behaviour of the plants of the second series it soon becomes evident that the preliminary treatment of plants by constant illumination retards flowering when the plants are transferred to normal day conditions, this retardation being more or less proportional to the number of days without darkness given to the plant. That is to say, no processes leading to reproduction will commence until plants are exposed to the alternation of light and darkness under normal day conditions, thus introducing the necessary quota of darkness.

The table shews that the number of normal days which the plants under trial require for flowering decreases. From this it is argued that no inhibiting factors are produced under long-day conditions, otherwise the plants which received a larger number of continuous days should shew delayed flowering.

" The decrease in the number of normal days required for the exsertion of panicles remains incomprehensible if we assume that the factor which makes impossible the preparation on the part of the plant for reproduction is the continuous illumination. If this were so the number of normal days should be actually the same for all plants. If, however, it be considered that plants grown invariably under continuous illumination (daylight plus electrical illumination which is not so intensive as sunlight) will finally flower, it becomes evident why variants in each trial require not the same number of normal days, but less in proportion to the number of continuous days received." " The uninterrupted day is itself the ' brake ' in the progress of millet towards reproduction. For the transition of the plant to reproduction, darkness is necessary and each light period will only retard this process by a time interval dependent on the intensity of the illumination."

The second trial was conducted in order to ascertain whether any after-effect of long day exists. Pots were sown in pairs at intervals of five days under continuous light. After four such sowings had been made, an interval of five days was allowed to elapse, after which all pots were exposed to the influence of normal day. Hence the series contained plants which received in the early stages of development 5, 10, 15 and 20 continuous days, respectively. If the after-effect of long day did exist, as described by Rasumov, the plant which received the smallest number of continuous days would be expected to flower earlier than those which received a larger number. This was not the case, all plants flowering on the same day, irrespective of the number of continuous days they had previously received.

Summarizing the results obtained in his trials, Dolgushin reaches the following conclusions :—

(1) " The action of a reduced length of day on millet in the earliest phases of its development is equivalent to the action of darkness on the process of preparation of the plant for reproduction.

(2) The results of this action may be manifested later when short-day plants are exposed to the influence of long-day conditions ; this might be called after-effect.

(3) No after-effect of long day when applied at an early stage is observed when the plants are subsequently grown under short day, and hence no substances inhibiting reproduction are produced by long day."

These considerations led Lyssenko to a new conception of the phenomenon of photoperiodism (13) and to the conclusion that the need for photoperiodism is not inherent in the nature of plants. " Photoperiodism exists," says the author, " but not in the nature of plants so much as in the nature of the regions where there occurs the daily alternation of day and night." Lyssenko is led to disagree with the common interpretation of photoperiodism which stresses the necessity for an alternation of light and darkness. Even in the well-known experiment of Garner and Allard (9) in which two soya bean varieties flowered when grown under alternating periods of 12 hours light and 12 hours darkness, on the 34th and 21st day respectively, when the alternation of light and darkness was done every hour, every minute and every 15 seconds, these plants did not flower, in spite of the fact that they received their quota of 12 hours of light and 12 hours of darkness per day.

It is pointed out that the so-called long-day plants reach the stage of reproduction most rapidly of all when grown under continuous illumination—i.e., when there is no alternation whatever. Such plants, it is stated, are more properly to be regarded as " plants of continuous light," merely tolerating the alternation of light and darkness and at that only when the dark period is short. Although in the case of short-day plants the necessity for alternation is more difficult to disprove, Lyssenko claims that in these also it is equally unnecessary.

Lyssenko's fundamental thesis is that the processes of "*growth*," as exemplified by gain in dry matter content, and the processes of "*development*," or the transition from one stage to another of the reproductive cycle, are two distinct phenomena and not necessarily antagonistic one to the other. Consequently, it is possible to influence one of these processes without of necessity interfering with the other. The plant may be subjected for a certain period to the influences which favour its reproductive development, during which time its growth is temporarily kept in check, and may later be transferred to other conditions favouring growth; in this way the two sets of processes are duly accelerated. The marked advantage of this method over previous methods based on photoperiodism alone is illustrated by the fact that in the Odessa experiments an accelerated reproductive development was accompanied by a vegetative development actually in excess of that of the control plants grown from ordinary untreated seed. If an antagonism between vegetative and reproductive development existed, the method of vernalization could not be applied to forage crops, since, as Lyssenko points out, there would be no advantage in attempting to obtain an earlier first cut if the yield were to be inferior to that of untreated plants. The reduced vegetative development in the earlier experiments is regarded as the result of reduction in the number of hours available for photosynthesis rather than a direct result of the accelerated reproduction, which latter is moreover ascribed not to the weak vegetative development, but to the influence of the long night which is received incidentally. From this point of view the long day of northern regions is regarded as an advantage, if it can be compensated for by darkness artificially supplied before sowing.

Lyssenko explains his postulates in the following words :—

(1) " The development of annual seed plants consists of a sequence of qualitatively different individual stages. By stage of development of a seed plant we understand only those qualitative changes occurring in the plant, or in its individual organs, without which it is impossible to obtain seed from sown seed. The remaining qualitative changes which might occur in a plant or in its organs and without which it is still possible to obtain seed from seed sown, we do not consider as stages of development. Not all stages of development which occur in a plant necessarily produce external morphological changes, and vice versa not all morphological changes observed in a plant are necessarily the result of the progress of that plant towards a new stage.

(2) " By growth of a plant we understand in our work the increase in the weight and size of a plant at any given stage of development. The growth of the plant is different at different stages of development.

(3) " The set of external conditions required by a plant for the completion of the *stages of development* and that required for *growth* frequently do not coincide, not only in the sense of the dose of factors, but also in the sense of the factors themselves. A plant may grow for an indefinite period, increasing in weight and size, under the influence of appropriate media and yet not progress towards the next stage of development. Thus winter cereals, when sown in spring grow (increase in weight), but they do not pass into the stage of reproduction until late autumn. In this case the progress towards the next stage (the stage of vernalization*) is retarded by high temperature. On the other hand, millet, soya bean and other short-day plants when grown under constant illumination do not pass into the reproductive stage because of the absence of darkness, whereas their growth may continue without interruption.

(*) Lyssenko uses vernalization here in the sense of a phase of development and not the method.

(4) " The rate of plant development, i.e., the rate of passage through the stages of development, does not depend on the rate of plant growth, i.e., on the rate of accumulation of dry mass or increase in size of the plant.

(5) " The plants at different stages of development may require different external conditions. The external conditions corresponding to one stage of a given plant may not at all correspond to another stage of the same plant.

" If the external conditions are favourable to all stages of development except one particular stage, then the plant when it approaches this particular stage ceases further development in spite of the fact that conditions are favourable to subsequent stages. In the individual development of a plant there is a strict sequence of stages of development. Without passing through a preceding stage of development, the plant cannot proceed to subsequent stages. Winter wheat, therefore, fails to ear, not because of high temperature in spring and summer generally, but because that temperature is too high for passing through the stage of vernalization. Without passing through that stage the wheat cannot proceed to the next, and hence reproduction does not occur in such sowings. Vernalized spring sowings of winter wheat reproduce normally under long-day conditions, but when grown under short-day conditions will not reproduce, the cause in this case being the short day, which affects not the wheat plant in general, but only one particular stage which is subsequent to that of vernalization. For this stage, wheat requires continuous illumination and can only tolerate the alternation of long day and short night, while for all other stages of development constant illumination is not obligatory. Winter wheat, when it has passed the stage of vernalization and the stages which requires constant illumination combined with other factors, may pass through all other stages up to the formation and ripening of seed, under conditions both of increased temperature and short day."

The same is true for so-called short-day plants which at one stage of development do not require light at all, but on the contrary require continuous darkness combined with the other necessary factors. This stage can also take place with a certain amount of alternation of light and darkness (long night and short day). All other stages, at least in such plants as soya bean and millet, may be passed not only under long day conditions, but even under constant illumination. Hence such plants as millet, soya bean and Sudan grass, etc., are in Lyssenko's opinion, incorrectly termed short-day plants ; it will be more correct to call them plants which require for one of their stages of development the absence of light, of course combined with the other necessary factors.

" In order to pass through any stage of development a definite and frequently considerable period of time is required. Thus for complete transition of the stages of development in millet, darkness or absence of light is required for 10-15 days according to variety and environment (temperature, humidity, etc.). Under field conditions where these plants are cultivated, continuous darkness for 10-15 days does not exist. Again, under artificial conditions, it is impossible to keep green plants in darkness for 10-15 days at a high temperature (which is necessary in this case) for this will destroy the plants. Although the " short-day " plants do require continuous darkness at a certain moment of their development, yet they also will tolerate a certain alternation of dark and light periods (long night and short day).

" For this reason it is possible to cultivate the so-called " short day " plants and " long-day " plants only within certain geographical zones, namely, short-day plants in southern latitudes where in spring and summer the day is short and the night is long, and long-day plants in northern latitudes where the day is long and the night is short.

" When it is possible to supply plants with dark periods of any duration (10-15 days) under artificial conditions, the plants may starve owing to the absence of photosynthesis. If a plant at the stage of newly germinated seed be placed under such conditions (under darkness), the seed may be nourished by the deposits in the endosperm and not through photosynthesis. If in addition a medium

be created for these plants (sowing material) which retards growth and favours those stages which require darkness, combined with other factors, it is a simple matter to force the plant to pass through the stage in question even before sowing.

" When the stages which require darkness are passed, the plant obtained from such treated seed can be grown not only under long-day conditions, but even under constant illumination ; hence neither " long " nor " short-day " plants require photoperiodism. For growth and development of " short-day " plants light and darkness, but not the alternation of short day and long night, are necessary."

In these articles no attempt is made to give a critical review of the other concepts of the theory of photoperiodism ; the division of plants into the three clearly defined groups is granted, although Lyssenko regards such a division as undesirable. The most recent experiments at Odessa indicate that it is rather a case of the requirement of various plants of different intensities of illumination during one period of their development.

V.—ARTIFICIAL RETARDATION OF GERMINATION.*

During the process of vernalization seeds are frequently kept moist for a considerable period. Although the temperature is relatively low during the vernalization process, seed germination, owing to an adequate moisture supply, progresses so vigorously that it may complicate subsequent sowing of overgrown seeds. It is necessary, therefore, to influence seeds so as to retard their growth and yet not damage their vitality in doing so. This may be effected either by physical or chemical means.

Reduction of moisture, which retards seed growth, is one of the physical methods. If this process be carried to excess, however, seed germination will be arrested. The appropriate minimum of moisture varies in different plants. Numerous observations have shewn that seed-swelling progresses while in water under an optimum temperature (20-25°C.) as follows :—

During the first four hours it progresses very vigorously, but during the next 12 hours water absorption is carried on at a gradually diminishing rate and finally seed swelling ceases. Experimental data shew that to retard seed growth 47-50 per cent. and 105-110 per cent. of humidity are required by wheat and soya bean respectively ; this amount is absorbed by them in four hours, whereas the 50 per cent. humidity required for cotton is absorbed under the same conditions in 24 hours. It has been assumed, therefore, that the difference both in the dose and in the rate of absorption depends partly upon the composition of the seed and partly on the permeability of the seed coat. At a low temperature water absorption progresses much more slowly ; thus 47-50 per cent. of humidity is taken up by wheat seed during 6, 12 and 18 hours, while under a temperature of 15-17°C., 10°C., and 5°C., respectively. A conspicuous difference in water absorption has been observed also between seeds which were previously swollen and then dried, and those which were untreated ; the former absorbed a larger proportion at a higher rate when compared with the latter.

Although modern literature contains little data on the effect of various salt solutions on seed swelling, there is a general consensus of opinion that the amount of water absorbed by a seed decreases as the concentration of such a solution increases. An inverse relation between the concentration of solutions and seed swelling was traced by the author in her experiments with chlorides and phosphates of potassium, sodium, ammonium, etc., and with balanced solutions of magnesium chloride and calcium chloride.

In the practice of vernalization, physical methods are usually employed consisting in limiting the amount of moisture available to the seed. The defect of this method is that the seed material may easily suffer from desiccation, in which case the same temperature and duration of treatment will not bring about complete vernalization. Chemical methods are for this reason to be preferred.

The basis of the experiments was that all salts, whether nutritive or poisonous, would necessarily cause poisoning when taken in excess. The same salts when applied in very low concentrations may be neutral or may even stimulate growth. It was necessary, therefore, to find an intermediate stage between the two extremes in which seed growth would be only retarded. Poisonous salts such as salts of heavy metals are unsuitable, as the neutral stage between harmless and poisonous concentration is extremely limited. The first trials, therefore, were concerned only with neutral salts of potassium, magnesium and ammonium, only the chloride anions being employed.

The procedure was as follows :—Seeds were soaked in the solutions to be tested for 24 hours ; the solutions were then poured off and the swollen seeds were placed in humid chambers ; four or five days later the seeds were sown and their germinability recorded. Although a concentration

(*) Bassarskaya (4).

that would only hinder growth could be selected, yet a prolonged action of the same salts might eventually be adverse. In later trials, therefore, the ordinary solutions were replaced by balanced solutions in which the poisonous action of the salts was mutually neutralized.

The only salts which do not damage the living organism when used in this way to balance sodium chloride are those of calcium and magnesium. Hence the author devoted her attention mainly to these salts.

Seeds of winter wheat were soaked for 24 hours in small crystallizing dishes, the solution to be tested being added in such a way as to submerge the seed (approximately 10 ccm. per 5 gr. of wheat seed). An excess of solution is advisable since bacteria and spores are washed off the seed coats, and also since it was found that seeds absorb a much larger amount of water than of salts, with the result that the concentration of the solution will gradually increase as the absorption progresses, thus eventually adversely affecting seed germination. Preliminary trials were conducted for 10 days at the summer room temperature of 22-23°C. in test tubes 6mm. in diameter or in small crystallizing dishes. Later trials were conducted in thermostats at a temperature appropriate for the vernalization of the seed under trial.

The first series of trials involved solutions of single salts such as KH_2PO_4, KCl, KNO_3, $(NH_4)HPO_4$, $(NH_4)_2PO_4$ and some others.

The second series of trials in which mixtures of salts were used may be subdivided into two parts.

(1) Seed treated with balanced solutions.

(2) Seed treated with buffer mixtures.

Sodium chloride to which a small quantity of calcium chloride had been added was the first balanced solution to be investigated. Low concentrations of sodium chloride, balanced with calcium and potassium, caused insignificant retardation in growth over a period of 8-10 days. Even solutions of somewhat higher concentrations were ineffective for wheat, although acting efficiently on cotton seeds. The growth of cotton seed was inhibited for 10-15 days when treated with balanced solutions in spite of appropriate humidity. These seeds when sown in the field germinated in 3-4 days and developed normally.

The results of trials with sea water were not decisive owing to the frequent changes occurring in its composition. In later trials, therefore, sea water was replaced by Van't Hoff's mixture diluted in certain ratios. In the case of cotton these solutions, more especially with low concentrations, gave quite satisfactory results. They were, however, unsuitable for wheat, the seed losing the capacity to germinate. Van't Hoff's mixture, therefore, was simplified, viz., only sodium chloride, magnesium chloride and magnesium sulphate were employed. The treated seeds began to germinate only after 10 days.

Further solutions composed of salts in which the cations were bivalent and balanced (calcium chloride and magnesium chloride) were also tested. Seeds treated with low concentrations of such salts grew slowly during the first days, after which their behaviour was similar to that of the controls. Seed treated with high concentrations developed much more slowly, and sometimes, as in the author's trial, the tip of the seedling only just appeared. For practical purposes, salts obtained by evaporating sea water should be the most suitable. The results of trials conducted over a 40-day period shew that concentrations of from 3.25-3.50 per cent. seem to be most suitable. Seeds thus treated germinated slowly and when sown their seedlings grew and developed normally. Seeds treated with low concentrations (2.75 per cent.) resisted very prolonged drying better than others. All types shewed more or less resistance to 10 days' drying, but only seeds treated with 2.75 per cent. concentration were thoroughly resistant to 15 days' drying.

As mentioned above, buffer mixtures with various pH values were also tested. The pH value is undoubtedly of great importance for growth ; on the other hand certain buffer mixtures retard the rate of enzyme action and it may be assumed that the growth rate of seed treated by these mixtures will be decreased according as the enzyme processes are reduced.

The following types of buffer mixtures were tested :

(1) sodium acetate with acetic acid ;

(2) (a) mono- and (b) di-potassium phosphate ;

(3) (a) mono- and (b) di-sodium phosphate ;

in the ratios adopted by Sorensen, but in somewhat higher concentrations. The trials with sodium acetate with acetic acid shewed negative results, the treated seeds losing their germinating ability. Different results were obtained with the other two mixtures. It ought to be mentioned that, in general, solutions of mono-potassium phosphate in concentrations varying from 3-4 per cent. retard seed growth and seeds when sown develop more or less normally.

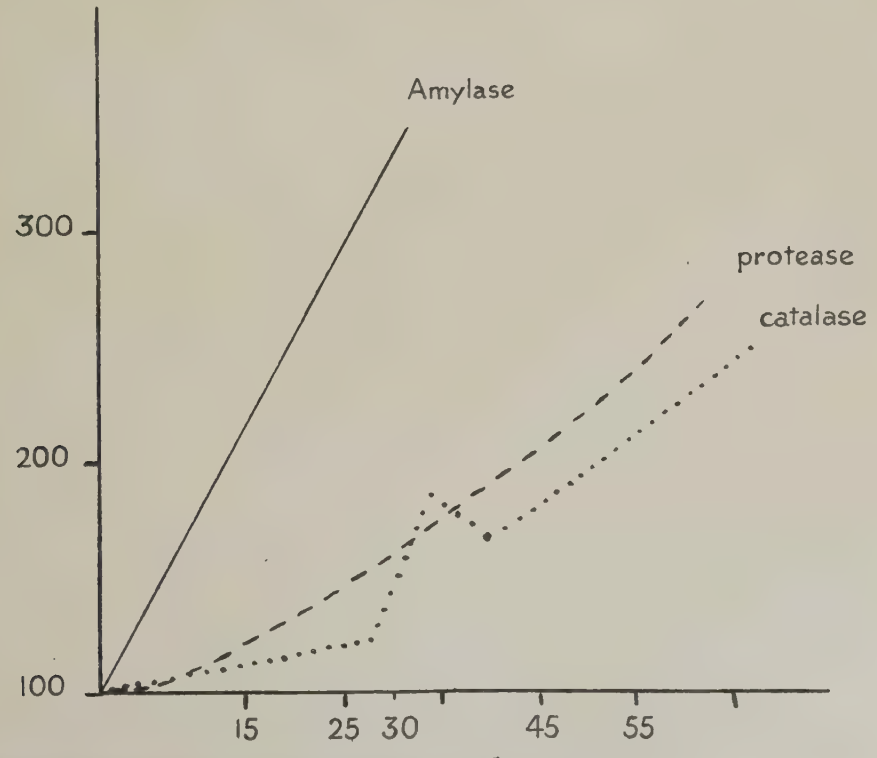
The results shewed that 0.257 mol. solutions of KH_2PO_4 and K_2HPO_4 gave the best results. The buffer mixture of the 3rd type is less suitable, as seed thus treated did not resist prolonged drying after vernalization ; 25 per cent. of the seed lost their germinating capacity in 10 days and only 20 per cent. were still able to germinate after 15 days' drying. In addition the di-sodium salt is soluble only at a high temperature, crystallizing out at a somewhat lower temperature.

If chemical substances are used for the retardation of seed growth during vernalization, it is necessary to note the influence of these substances on flowering and ear formation. Results obtained up to the present shew that at least the above mentioned salts do not hinder flowering.

VI.—BIOCHEMICAL ASPECTS OF VERNALIZATION.

Following on the above described experiments on vernalization, Demkovsky (5 and 6) has made studies of the enzyme action occurring during vernalization. From the fact established by Lyssenko that the vernalized plant develops more dry matter per unit of time than an untreated plant, it was assumed that the enzyme activity is higher in the vernalized plant. Biochemical differences between early and later varieties in their enzyme complex might also be expected.

The preliminary investigation of vernalized seeds in which the amylase, catalase and protease were studied, has confirmed this conception : enzyme activity was increased by vernalization and the rate of activity was different for amalyse, catalase and protease. That is to say, both a general increase of enzyme activity and also a change in the interrelation between different groups of enzymes occurred. The analysis shewed that the enzyme complex (amylase and catalase only) of vernalized seeds, which owing to a certain delay in sowing had lost their germinating capacity, was found to be balanced, irrespective of the degree of vernalization. The material of this preliminary study consisted of seed of winter wheat (Stepniachka) harvested in 1930 and vernalized according to Lyssenko's method. The samples were taken during treatment on the 15th, 25th, 30th, 35th and 45th day from the beginning of vernalization, and subjected together with the control (untreated) seeds to analysis with respect to enzyme complex, viz., amylase, catalase and protease.



Graph of changes in enzyme activity during vernalization (after Demkovsky, Bull. Yarovizatzii. No. 2-3. p. 106.)

The change in pH of aqueous extracts taken from seeds at various stages of vernalization was also studied.

The following questions had to be decided : (1) whether the activity of the enzymes should be judged by working with the enzymes in their own or in a foreign substrate, and (2) whether to apply buffer solutions or not. It was decided to work with enzymes in their own substrate without the addition of buffer solutions since " (1) in this case we deal with the same variety, and (2) if vernalized seed (substrate) undergoes during vernalization any changes which can influence enzyme activity, these changes might be annulled when the enzymes act on a foreign substrate or when buffer mixtures are added. It was decided, therefore, to introduce a control medium (determination of pH) on the one hand, and on the other hand to carry out preliminary analysis on a foreign substrate or with the addition of a foreign substrate, and to carry out preliminary investigation with the addition of buffer solutions."

As may be seen from the foregoing diagram, enzyme activity increases as vernalization progresses. During vernalization the processes connected with seed germination may also be proceeding slowly. The changes observed in the enzyme complex may reflect these processes and take place parallel with the process of vernalization. This furnishes an indirect method of determining the degree of vernalization of the seed. The study of this question is in progress. There are also reasons for supposing that in addition to temperature, other facts such as darkness have an influence on vernalization.

While making the necessary reservations on the score of the incompleteness of the data, Demkovsky for the time being concludes that enzyme activity gradually increases during vernalization, catalase up to 30 days, amylase up to 25 days, and protease throughout the process of vernalization.

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